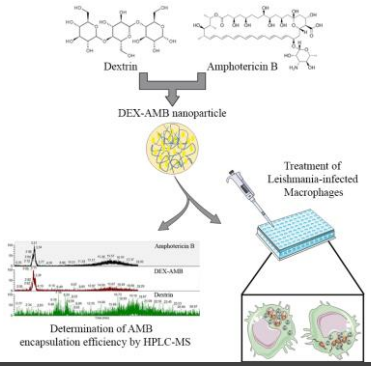
Development of a water-soluble dextrin-amphotericin B conjugate for the treatment of Leishmaniasis

*J. Fidalgo[1, 2 *], R. Silva-Carvalho[1*], S. Leal[1], T. Cruz[2], AM. Tomás[2], P. Parpot[1, 3], M. Gama[1]*
[1] CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; [2] I3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto and IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; [3] Centre of Chemistry, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.
** remanuelcarvalho@gmail.com*



Leishmaniasis is a life-threatening disease with a worldwide distribution and a huge impact especially in immunocompromised patients. Nowadays, the use of amphotericin B, an antifungal agent, or other marketed strategies to overcome this disease exhibit major clinical limitations. Thus, other strategies have been pursued to achieve better clinical treatments and decrease toxicity. In this work, a promising conjugate based on dextrin and amphotericin B was developed. An HPLC-MS detection method was also developed in order to determine the amphotericin B concentration and the encapsulation efficiency. This water-soluble polymer-drug conjugate has shown promising effects against axenic cultures of *Leishmania amazonensis* parasite and infected macrophages, without being cytotoxic to the latter.

Leishmaniasis has been classified as one of the most neglected tropical diseases that affects around 2 million people worldwide (with an incidence of about 0.5 million cases annually) causing morbidity and mortality [1]. This disease, which is endemic in poor countries, is also found in Europe (transmitted as a zoonosis). In fact, there are 12 million people currently infected globally and 350 million people in 88 countries all over the world living at risk of developing one of the many forms of the disease [1, 2]. Protozoan parasites of the genus *Leishmania* are responsible for this life-threatening disease. These parasites can maintain their life cycle through transmission between two forms: the extracellular motile promastigote found in insects (sandfly) and the intracellular non-motile amastigote inside the macrophages of mammalian hosts [3]. Similarly to other intracellular pathogens, such as *Mycobacterium tuberculosis*, this life-threatening parasite can cause a range of diseases, especially in immunocompromised patients [1, 3]. More specifically, some *Leishmania* species (e.g. *L. major*, *L. mexicana*, *L. amazonensis and L. braziliensis*) promote cutaneous, mucocutaneous or diffuse cutaneous leishmaniasis, characterized by localized symptoms. Other *Leishmania* species that are more aggressive (e.g. *L. donovani*, *L. chagasi*, *L. infantum)*, target internal organs causing visceral leishmaniasis. This form of the disease is responsible for approximately 70,000 deaths per year and is associated to different symptoms: fever, weight loss, splenomegaly, hepatomegaly and anemia [2, 3].

Despite the well-established knowledge and the recent advance in our understanding of leishmanial biology, some aspects of this disease remain enigmatic and because of that the current control or treatment strategies are rather inadequate [3, 4]. This is either due to limited availability of effective parenteral drug formulations or constant appearance of new fungal infections, which are resistant to the available drugs on the market. Besides, as there are currently no effective vaccines to prevent human leishmaniasis, the management/cure of the disease relies on chemotherapy, where the drugs are of high toxicity, low efficacy and difficult to administer [2, 5]. Amphotericin B (AMB) - a highly toxic water-insoluble compound - is a polyene antibiotic used as standard drug for fungal infections. Currently it is recommended as a second-line treatment for visceral and mucocutaneous leishmaniasis. Nevertheless, this therapy is limited since AMB is difficult to solubilize, promote side effects (nausea, fever and chills) and is toxic, mainly to the kidneys, central nervous system and liver. Considering that, strategies such as the use of combination therapy, modification of the AMB molecule, modification of the physical state of AMB and use of drug delivery system (liposomal formulations, lipid complexes, lipid emulsions, colloidal dispersions, among others) have been the cornerstones to improve the therapeutic efficacy and to reduce the toxicity of AMB, even at high doses. However, there are some lipid products (e.g. AmBisome) or micellar formulations (e.g. sodium deoxycholate AMB - Fungizone) that, despite being on the market, exhibit major clinical limitations [5, 6].

The use of proteins, polypeptides, polysaccharides and synthetic polymers to achieve water-soluble polymer-drug conjugates has attracted considerable attention in recent years since it may enable drug targeting while reducing drug toxicity [5, 7]. Different polymeric drug carriers have been widely studied for this purpose, including polysaccharides [5, 7, 8], which possess high water-solubility, low toxicity, a high degree of biodegradability and biocompatibility [9].

In this work, we hypothesize that dextrin may be an interesting polysaccharide for the development of a drug delivery system as it is a biocompatible and nonimmunogenic material, degradable *in vivo* by amylases, making it a potential asset for use in the biomaterials field [10]. Considering that, the purpose of this work was to achieve and characterize a new water-soluble dextrin-amphotericin B (DEX-AMB) conjugate and test its efficacy against Leishmania infection.

The conjugate, which was obtained by mixing dextrin with AMB, was characterized in terms of size/morphology by cryo-SEM. Furthermore, an HPLC-MS detection method was optimized and used to determine the AMB concentration and the encapsulation efficiency (EE) in the conjugate. Leishmanicidal activity of the DEX-AMB was assessed *in vitro* in axenic cultures of *Leishmania amazonensis* by resazurin and infected bone marrow-derived macrophages stained with different fluorescent probes using high-content microscopy (IN Cell Analyzer 2000). The cytotoxic effects of the conjugate were assessed on bone marrow-derived macrophages by resazurin.

In terms of size/morphology characterization, cryo-SEM analysis has shown that this conjugate has the ability to form spherical particles in aqueous solution. Those particles are within the nanometric size range, with a hydrodynamic diameter of around 100 - 200 nm. This analysis also prove that the conjugate is soluble in water, since no signs of drug precipitation were observed.

As previously stated, the development of an HPLC-MS quantification method was very important in this work, since it

has been found that the commonly used methods, such as spectrophotometry or HPLC-UV, are not reliable. In this work, a discussion on the limitations of the commonly used methods for estimation of AMB will be provided and a new method is proposed.

In vitro assays were performed to evaluate possible cytotoxic effects against bone marrow-derived macrophages and to assess capacity of the DEX-AMB to reduce the parasite infection. Comparatively to the non-encapsulated AMB, the conjugate material showed less cytotoxicity at the higher tested doses. In the *L. amazonensis*-infected bone marrow-derived macrophages, all the tested doses of our conjugate promoted a slightly higher inhibition of the infection comparatively to the non-encapsulated drug.

In conclusion, this work has shown that DEX-AMB is a promising drug delivery system for the treatment of Leishmaniasis. This is proved by the capacity of our polymer-drug conjugate to generate similar effects to the ones obtained with non-encapsulated AMB against Leishmania-infected macrophages and Leishmania axenic cultures, with the advantage of being significantly less cytotoxic.

Acknowledgements

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte. Ricardo Silva-Carvalho gratefully acknowledge the Portuguese Foundation for Science and Technology (FCT) for the granted scholarship (SFRH/BD/118880/2016).

References

[1] S. Vale-Costa, S. Gomes-Pereira, CM. Teixeira, G. Rosa, PN. Rodrigues, A. Tomas, R. Appelberg, MS. Gomes, PLoS Negl Trop Dis, 7 (2013), e2061.

[2] L. Kedzierski, J Glob Infect Dis, 2 (2010), 177-185.

[3] P. Kaye, P. Scott, Nat Rev Microbiol, 9 (2011), 604-615.

[4] M. Oliver, Nature, 471 (2011), 173–174.

[5] T. Ehrenfreund-Kleinman, T. Azzam, R. Falk, I. Polacheck, J. Golenser, AJ. Domb, Biomaterials, 23 (2002), 1327-1335.

[6] J. Brajtburg, J. Bolard, Clin Microbiol Rev, 9 (1996), 512-531.

[7] T. Ehrenfreund-Kleinman, J. Golenser, AJ. Domb, Biomaterials, 25 (2004), 3049-3057.

[8] M. Sokolsky-Papkov, AJ. Domb, J. Golenser, Biomacromolecules, 7 (2006), 1529-1535.

[9] DE. Ickowicz, S. Farber, E. Sionov, S. Kagan, A. Hoffman, I. Polacheck, AJ. Domb, Biomacromolecules, 15 (2014), 2079-2089.

[10] DM. Silva, C. Nunes, I. Pereira, AS. Moreira, MR. Domingues, MA. Coimbra, FM. Gama, Carbohydr Polym, 114 (2014), 458-466.